

THE EFFECT OF CHEMICALS AND
TEMPERATURE ON REVERSAL
IN CILIARY ACTION IN
PARAMECIUM

A DISSERTATION SUBMITTED TO THE BOARD OF
UNIVERSITY STUDIES OF THE JOHNS HOPKINS
UNIVERSITY IN CONFORMITY WITH THE
REQUIREMENTS FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

By

JOSEPH F. OLIPHANT

1937

Reprinted for private circulation from
PHYSIOLOGICAL ZOÖLOGY, Vol. XI, No. 1, January, 1938

THE EFFECT OF CHEMICALS AND
TEMPERATURE ON REVERSAL
IN CILIARY ACTION IN
PARAMECIUM

A DISSERTATION SUBMITTED TO THE BOARD OF
UNIVERSITY STUDIES OF THE JOHNS HOPKINS
UNIVERSITY IN CONFORMITY WITH THE
REQUIREMENTS FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

By

JOSEPH F. OLIPHANT

1937

Reprinted for private circulation from
PHYSIOLOGICAL ZOÖLOGY, Vol. XI, No. 1, January, 1938



THE EFFECT OF CHEMICALS AND TEMPERATURE ON REVERSAL IN CILIARY ACTION IN *PARAMECIUM*¹

(One figure)

JOSEPH F. OLIPHANT
Stanford University

REVERSAL in direction of the effective stroke of the cilia is very common in the protozoa and occurs frequently in the metazoa, but only certain phases of it have been adequately investigated, and few attempts have been made to ascertain the physiological and environmental factors involved in the phenomenon.

Jennings (1899a) found that a variety of stimuli (chemical, mechanical, electrical, and thermal) cause *Paramecium* momentarily to reverse its direction of locomotion. He further noted (1899, p. 34) that in paramecia put into a 1 per cent solution of KI the action of the cilia reverses for 6-8 minutes.

Parker (1896, 1905a, 1905b) found the action of the cilia on the oral disk of *Metridium marginatum* reverses in response to dilute crab-meat juice, a sea-water extract of rubber, 2, 3, 4, and 5 per cent solutions of KCl in sea water, a $5/8$ M solution of NaCl containing M/15 or M/30 creatin, to peptone or deuto-albumine, and asparaginic acid. He did not obtain reversal using NaCl alone; LiCl, NHCl, NaNO₃, a number of sugars, urea, creatinine, or chemically inert substances such as bits of filter paper or sand. Before he had worked with organic compounds other than crab-meat juice, he said (1905a, p. 13): "Reversal is due to potassium ions or to some organic combination containing potassium ions." After having observed the effects of various organic compounds, he modified these statements and said (1905b, p. 5): "The reversal . . . is due chiefly to molecular action, though potassium ions if sufficiently concentrated will cause reversal."

Merton (1923) obtained reversal of ciliary action in *Stentor coeruleus* in solutions containing Na and K ions but did not obtain reversal in solutions of CaCl₂, MgSO₄, and MgCl₂. He suggested that the reversal is due to the Na and K ions and concluded (p. 13):

Wenn der Zusatz von K oder Na Ionen eine Umkehr der Flimmerbewegung hervorruft, so besteht die Möglichkeit, das unter natürlichen Bedingungen diese Ionen selbst in dem Stentor wirksam sind. Es kann aber auch und das ist wahrscheinlicher ein unbekannter Stoff diese Reaktion hervorrufen und nur im Versuch durch diese Kationen ersetzt werden.

Merton (1935) found in further studies with *Stentor* that a number of salts of K, Na, Rb, Ce, and NH₄ induce reversal in the action of the cilia. Reversal, however, was not obtained with calcium salts. He says that reversal results from some specific action of the alkali metals, though he does not say what the specific action is.

Mast and Nadler (1926) investigated the relation between fifty-six different chemicals in the environment and reversal in ciliary action in *Paramecium caudatum*. By observing the direction of locomotion, they ascertained qualitatively the effect of the chemicals

¹ Contribution from the Zoölogical Laboratory, Johns Hopkins University. The author wishes to acknowledge his indebtedness to Professor S. O. Mast for valuable suggestions concerning the experiments and for valuable assistance in the preparation of the manuscript.

in inducing reversal. They found that all the univalent salts and hydrates tested induce reversal except $(\text{NH}_4)_2\text{SO}_4$ and $(\text{NH}_4)\text{C}_2\text{H}_3\text{O}_2$ but none of the bivalent and trivalent cation salts and hydrates except CaHPO_4 , MgHPO_4 and $\text{Ba}(\text{OH})_2$. Polyvalent cation salts were found to neutralize the effect of monovalent cation salts. They conclude (p. 117): "Ciliary reversal is associated with differential absorption of cations and subsequent changes in electrical potential, but there are also other factors involved."

Eisenberg-Hamburg (1932) reported a *Rückwärtsbewegungsreaktion* when *Paramecium* is introduced into solutions of salts of Sr, Rb, and Ce. She maintains that this reaction consists of a series of backward and forward movements extending over considerable periods of time and that the reversed phase is in each instance of very short duration. The reaction was also obtained, though not in such a characteristic manner, in Ba salts but was not obtained in Mg, Ca, Cd, Na, K, and Fe salts. Similar results were obtained with *Colpidium*, *Stentor*, and *Spirostomum*. She concludes (p. 123): "Die Rückwärtsbewegungsreaktion bildet somit eine Reflexbewegung des normalen Infusors, hervorgerufen durch die Anwesenheit des Sr Kations [and by inference other cations] dessen chemisch-physikalischen Wirkung aller Wahrscheinlichkeit nach die Kolloide der Zelle angreift."

It is the object of the present investigation to ascertain more precisely the relation between monovalent cation salts in the environment and reversal in the effective beat of cilia with the view of throwing light on the physiological factors involved in ciliary reversal.

MATERIALS AND METHODS

For this investigation *Paramecium multimicronucleatum* was chosen owing to its size and the readiness with which it lends itself to culture and experimentation. The specimens used were all obtained from a clone cultured as follows: 7 gr. of timothy hay in a liter of Chattolane spring water were heated and kept at the boiling-point for 3 minutes; then 200 cc. of this infusion were poured into each of five Erlenmeyer flasks, which were then stoppered with cotton plugs and left 12 hours, after which 5 cc. of old infusion containing paramecia were added to each flask. New cultures were prepared each week, and no culture was used for experimentation until after it had stood for a week. By this time the hydrogen-ion concentration of the culture was $\text{pH } 6.9 \pm 0.1$. The temperature of the room in which the cultures were grown varied between 21° and 23°C. , inclusive. By this method animals in nearly the same physiological condition were obtained for the experiments.

The chemicals used were Kahlbaum's "pro-analyse" and Merck's blue-label Reagent. The chemical solutions were made with Chattolane spring water. This spring water was found to be more favorable than any other solution used. Distilled water has been used by other investigators, but it is not as satisfactory as spring water, for, when *Paramecium* is transferred from a culture medium to distilled water, action of the cilia is reversed. It is therefore necessary to acclimatize specimens to distilled water before making tests of effects of chemicals. This acclimatization must be done by gradual dilution over a period of 12 hours or more and even then there are not infrequently produced ruptures of the ectosarc by the entrance of water.

Merton (1935) used tap water in his studies on *Stentor*. In preliminary experiments of the present work it was found that various tap waters and spring waters can be used but that few of them are satisfactory because of great seasonal variation in content.

Chattolane spring water is obtained from covered springs, and chemical analyses show that its variation in total salt content throughout the year is only about 1 milligram per cent.² It tends to act as a natural buffer, and it has no appreciable effect on *Paramecium*. Thoroughly washed specimens live in it for as long as 10 days.

In making the tests, paramecia were first thoroughly washed in spring water, transferred to pure spring water, and left for an hour before being used for experimentation. To ascertain the effects of chemicals on ciliary reversal, 2.5 cc. of solution to be tested were put into a Columbia pyrex glass culture dish on the stage of a dissecting binocular microscope. One of the washed paramecia was taken up in a capillary pipette, care being exercised to take as little water as possible, and dropped into the test solution. The specimen was then observed continuously until reversed action of the cilia ceased and the duration of reversal measured with a stop watch. This was repeated with other specimens as often as was desired.

Numerous experiments by various investigators demonstrate that reversal in the action of the cilia in *Paramecium* is sometimes confined to localized regions of the body. In all the experiments presented here ciliary reversal was only recorded as having occurred if the specimens actually swam backward for a time measurable with a stop watch.

The method described above was slightly modified in some experiments as indicated in the following pages.

RELATION BETWEEN CONCENTRATION OF MONOVALENT CATION SALTS AND REVERSAL IN CILIARY ACTION

The time of backward swimming was ascertained for ten specimens in each of a series of concentrations of the chlorides, iodides, and bromides of sodium, potassium, lithium, and ammonium. The hydrogen-ion concentration of the chemical solutions used was $\text{pH } 7.4 \pm 0.1$, that of the culture medium from which the organisms were taken $\text{pH } 6.9 \pm 0.1$, and that of the wash solution to which the specimens were adapted $\text{pH } 7.4$. The temperature varied between 21° and 23°C .

In molar solutions in NH_4Br , NH_4Cl , and NH_4I the specimens died immediately without reversal, while in all the other salts they died in 1-2 seconds and swam backward continuously. In $\text{M}/5$ and $\text{M}/10$ NH_4I death was immediate, and there was no reversal. In $\text{M}/5$ and $\text{M}/10$ LiI and LiBr death occurred in about 10 seconds, and the paramecia swam backward continuously. In $\text{M}/5$ concentrations of the other salts death occurred in 2-8 minutes, and there was an initial period of reversed ciliary action which was followed by momentary recovery and normal forward movement after which there were repeated reversals and recoveries until death. In $\text{M}/5$ NaBr , for example, the animals swam backward $9.45 \pm$ seconds then forward 3-5 seconds then backward 0.5-1.0 seconds then forward 1-3 seconds and then continued to reverse at this frequency about 2-3.5 minutes, i.e., until they died, which usually, but not always, occurred in a reversed phase of about 30 seconds' duration. This type of behavior occurred in the other sodium salts and in $\text{M}/5$ and $\text{M}/10$ LiCl . In higher concentrations the animals, immediately before they died, revolved in a plane about the posterior end without rotating on the longitudinal axis. In other concentrations of all salts tested the initial resumption of forward movement was seldom followed by subsequent reversals. Re-

² Composition of Chattolane spring water in grams of salt per liter: NaCl , 0.0034; Na_2CO_3 , 0.00013; K_2SO_4 , 0.0017; CaSO_4 , 0.00007; MgCO_3 , 0.020; CaCO_3 , 0.052; silica, 0.0092; oxides of Fe and Al, 0.00066.

covery from a reversed condition in these concentrations was always preceded by a type of behavior in which the anterior end of the animal moved in a great circle while the posterior end moved in a small circle. Forward movement in recovered specimens was a little slower than in animals not subjected to the action of a chemical.

In M/400 of all the salts tested except those of ammonium there was a pronounced motor response accompanied by momentary reversal of such short duration that it could not be measured with a stop watch. In M/1,000 of the various salts except those of ammonium paramecia gave a slight motor response, but in no instance was there anything that could be considered true ciliary reversal. Similar results were obtained in ammonium salts M/50-M/150.

The results of one complete series of experiments are summarized in Table 1 and Figure 1. The table and figure show that in solutions of chlorides, iodides, and bromides of sodium, potassium, lithium, and ammonium duration of reversed ciliary action varies directly with concentration of the salt and that the order of effectiveness of the monovalent cations in inducing ciliary reversal is $K > Li > Na > NH_4$. A typical series of readings from which the average duration of reversal was computed, the one obtained in M/10 NaCl, the first salt in the table, follows: 11.1, 10.8, 11.2, 12.5, 11.9, 12.0, 10.5, 10.1, 8.4, and 9.6 seconds. The variation in individual readings was somewhat greater in bromides and iodides of potassium and lithium. This is shown typically by the results obtained with M/10 KBr which are 28.2, 32.5, 28.8, 27.6, 27.9, 27.1, 44.3, 25.6, 37.5, and 36.1 seconds. These series indicate considerable variation; however, in columns 4, 5, and 6 of the table it is revealed that the standard deviation is small in comparison with the average time of reversed ciliary action and that the probable error is very small, rarely exceeding 6 per cent. The standard deviation and percentage of probable error are greatest in the iodides, less in the bromides, and least in the chlorides. The reason for this variation is not clear.

All the experiments summarized in Table 1 and Figure 1 were repeated twice, and the results obtained are qualitatively and quantitatively in agreement with those presented.

The conclusions of these experiments are in accord with the conclusion of Eisenberg-Hamburg (1932) that the *Rückwärtsbewegungsreaktion* is greater, the greater the concentration of the salt inducing it, and they are supported by the results obtained by Merton (1935) on *Stentor*, which indicate that duration of reversal varies directly with concentration; but they are not in accord with the results obtained by Mast and Nadler on observations on *P. caudatum*. They say (1926, p. 116):

The duration of reversed action varies with the kind of salt and with the concentration. In KCl as the concentration decreases, the duration of this action increases from zero at M/10 to a maximum of about 40 seconds at M/750, then decreases to zero at M/2000. In NaCl it increases from zero at M to a maximum of about 14 seconds at M/500, then decreases to zero at M/2200. In NH_4Cl it increases from zero at M/50 to a maximum of about 10 seconds at M/1000, then decreases to zero at M/2200. In LiCl it increases very rapidly from zero at M/5 to a maximum of about 55 seconds at M/10, then decreases slowly to zero at M/2000.

The results presented above are obviously not in accord with these claims, for they show that in solutions of KCl, LiCl, and NH_4Cl duration of reversed ciliary action varied directly with concentration and that no reversal in ciliary action was obtained in concentrations between M/500 and M/750, the optimum for reversal found by Mast and Nadler.

TABLE 1

RELATION BETWEEN DURATION OF REVERSED CILIARY ACTION IN *Paramecium*
AND CONCENTRATION OF MONOVALENT CATION SALT INDUCING REVERSAL

"Average," time of reversal for ten specimens; S.D., standard deviation; P.E., probable error; percentage P.E., percentage probable error of the average duration of reversal.

Salt	Concentration	Average	S.D.	P.E.	Percentage P.E.
NaCl	M/10	10.81	1.81	0.25	2.3
	M/20	7.49	0.80	0.16	2.1
	M/50	5.73	0.87	0.18	3.1
	M/100	6.04	1.40	0.29	4.7
	M/200	4.26	0.77	0.16	3.7
KCl.	M/10	43.82	3.03	0.64	1.4
	M/20	30.05	3.59	0.74	2.4
	M/50	14.22	3.56	0.74	5.2
	M/100	3.92	0.95	0.20	5.1
	M/200	1.00	Not calculated		
LiCl.	M/10	13.22	1.88	0.40	3.1
	M/20	9.00	1.24	0.26	2.8
	M/50	7.52	1.80	0.38	5.0
	M/100	5.28	0.86	0.18	3.4
	M/200	3.52	0.55	0.11	3.1
NH ₄ Cl.	M/5	21.20	2.25	0.47	2.2
	M/10	8.70	1.78	0.37	4.2
	M/20	2.10	0.38	0.80	3.8
	M/50	1.00	Not calculated		
	M/100	?			
NaBr.	M/10	8.12	1.73	0.33	4.0
	M/20	5.63	1.24	0.21	3.7
	M/50	3.74	1.11	0.25	6.6
	M/100	1.00	Not calculated		
	M/200	?			
KBr.	M/10	31.56	5.92	1.26	5.1
	M/20	29.78	8.62	1.80	6.0
	M/50	9.25	2.27	0.48	5.1
	M/100	3.14	0.78	0.16	3.9
	M/200	1.00	Not calculated		
LiBr.	M/10	8.59	1.53	0.26	3.0
	M/20	13.25	5.15	1.63	12.1
	M/50	5.71	0.20	0.13	2.1
	M/100	3.89	1.05	0.22	5.6
	M/200	1.00	Not calculated		
NH ₄ Br.	M/5	10.62	2.81	0.59	5.5
	M/10	3.97	1.65	0.34	8.3
	M/20	1.00	Not calculated		
	M/50	?			
NaI.	M/10	7.53	2.78	0.59	7.9
	M/20	4.74	0.70	0.14	2.9
	M/50	3.47	1.20	0.24	6.8
	M/100	2.95	1.02	0.21	7.1
	M/200	1.00	Not calculated		

TABLE 1—*Continued*

Salt	Concentration	Average	S.D.	P.E.	Percentage P.E.	
KI.. .. .	M/10	53.36	14.37	3.06	5.7	
	M/20	49.92	15.26	3.24	6.4	
	M/50	12.57	3.46	0.73	5.7	
	M/100	4.16	1.02	0.21	5.0	
	M/200	1.00	Not calculated			
LiI	M/10	14.09	3.95	0.84	5.2	
	M/20	13.95	4.25	0.90	6.4	
	M/50	6.02	1.95	0.41	6.8	
	M/100	4.43	1.70	0.36	8.1	
	M/200	1.00	Not calculated			
NH ₄ I.....	M/10	Death without reversal				
	M/20	5.35	1.35	0.24	4.4	
	M/50	8.22	1.03	0.21	3.2	
	M/100	6.49	0.97	0.20	3.0	
	M/200	?				

The differences in results of the two investigations may have been due to differences in physiological states of the organisms traceable to conditions in the infusions in which they were grown and to adaptation to different wash solutions. Mast and Nadler used *P. caudatum* adapted to distilled water. The hydrogen-ion concentrations were not controlled, and the temperatures are not given. The differences are not due to the fact that different species were used, for in tests of *P. caudatum* in KI, KCl, and KBr under conditions of the present investigation results were obtained which are essentially the same as those obtained with *P. multimicronucleatum*.

THE ROLE OF ANIONS IN CILIARY REVERSAL

The possible role of the anions used in the present experiments was considered. When the data presented in Table 1 are compared, it is found that the difference in effect produced by like concentrations of chloride, bromide, and iodide having the same cation is in all instances slight, and it is impossible to recognize a consistent order in which these anions affect ciliary reversal.

To investigate further the possible role of anions, the effect of some bivalent cation salts was tested. Calcium and magnesium were used because they are closely related chemically to the alkali metals, whose salts induce reversal, and they are among the more abundant bivalent cations found in and having pronounced effects upon various types of protoplasm. Chlorides, bromides, and iodides of calcium and magnesium were tested from M/1 to M/20,000. In concentrations from those not immediately lethal about M/250 up to M/2,500 of each salt various types of motor reactions were observed, but in no instance was there anything that could be considered true ciliary reversal. These experiments indicate that calcium and magnesium ions do not induce reversal in ciliary action in *Paramecium* and that chlorine, bromine, and iodine ions do not induce reversal and that their action in reversal is quantitative. No other anions were investigated. However, Merton (1935) in experiments on *Stentor* presents evidence which indicates that some salts of potassium are more effective in inducing ciliary reversal than others. He lists the anions used above in the order Cl > I > Br.

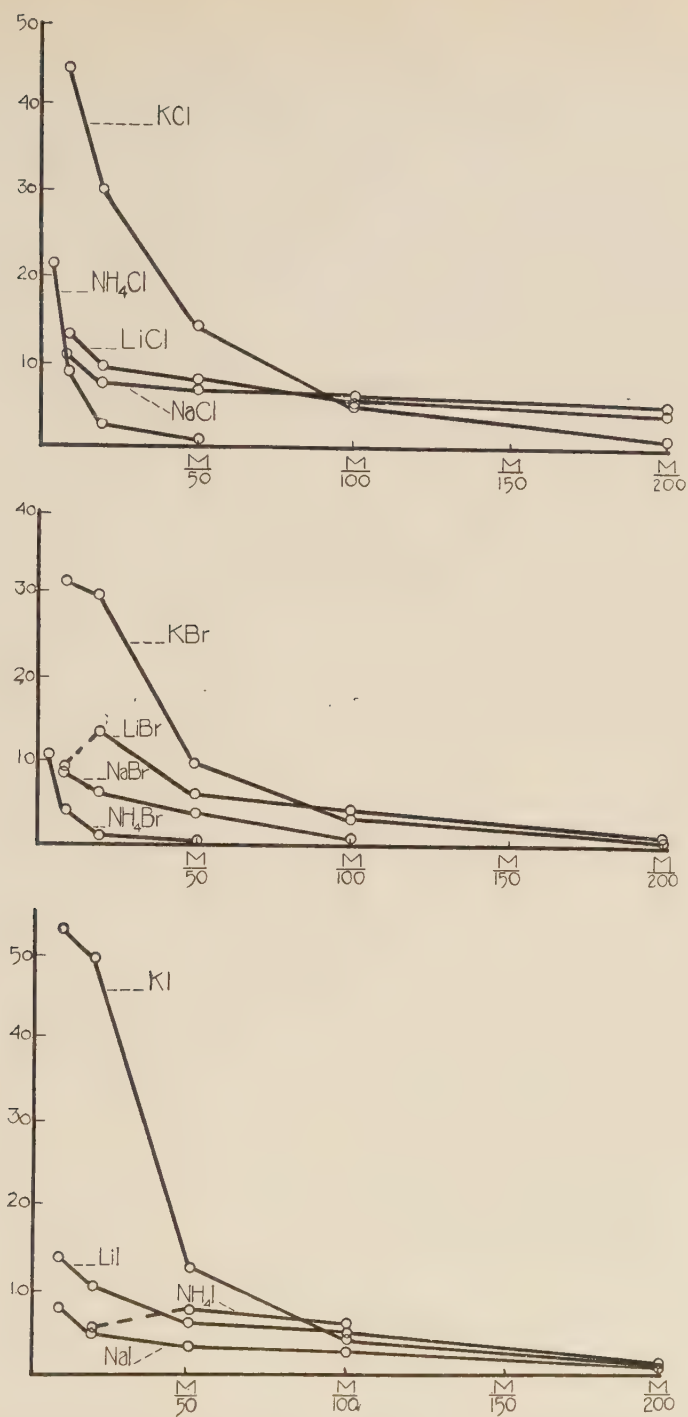


FIG. 1.—Graphs showing the relation between duration of reversed ciliary action in *Paramecium* and concentration of various monovalent cation salts inducing reversal. Ordinates, time of reversed ciliary action in seconds; abscissae, concentration of salt.

THE EFFECT OF ADAPTATION ON CILIARY REVERSAL INDUCED BY SOLUTIONS
OF MONOVALENT CATION SALTS

The question whether ciliary reversal in *Paramecium* is dependent on the absolute concentration of monovalent cation salt in the environment or upon rate of change (increase) in concentration has a direct bearing on adaptation. It was investigated as follows: Numerous paramecia were thoroughly washed in spring water; then 6 cc. of solution containing washed paramecia were added to 6 cc. of a monovalent cation salt solution M/20 in a 25 cc. stender dish. The dish was then covered and left for 18 hours. These paramecia and others which were washed but had not been in the salt solution were then tested for reversal in ciliary action in a number of concentrations of the salt to which the experimental animals had been exposed. This experiment was repeated with the chlorides, iodides, and bromides of sodium, lithium, and potassium. Care was exercised to adapt approximately the same number of organisms in each experiment.

It was found that *Paramecium* becomes adapted (no longer swims backward) to M/40 solutions of all salts tested in less than 18 hours, otherwise the adapted paramecia behaved essentially like those which were not adapted; however, the behavior was not the same in all salts. The paramecia adapted to lithium and potassium salts moved slightly slower and were darker and much more vacuolated than the controls. A microscopic examination revealed an apparent increase in protoplasmic consistency, and there was evidence of slight plasmolysis. Specimens adapted to sodium salts behaved more nearly like the controls; however, they were darker in appearance and more vacuolated. No difference could be detected between paramecia adapted to chlorides, iodides, and bromides having the same cation. After paramecia had remained two days and longer in lithium and potassium salts, movement was accompanied by active changes in shape, was very slow in comparison with controls, and reversals in the direction of rotation on the longitudinal axis increased. The paramecia in lithium salts died first, then those in potassium salts, then those in sodium salts. The order in which the cations produce death therefore seems to be in no way correlated with the order in which they induce reversal in ciliary action. Observations were not made at sufficiently frequent intervals to ascertain the order of the anions.

The rate of reversed beat of the cilia was observed to differ in the different salts, but no actual measurements were made. However, it could be seen that it was greatest in lithium, less in potassium, and least in sodium salts. This is not in accord with the order of effectiveness of these cations in inducing ciliary reversal, but it is in accord with the order in which they produce death.

Some of the results of the experiments are presented in Table 2. This table shows that in paramecia adapted to a M/40 solution of a given monovalent cation salt ciliary reversal does not occur in lower concentrations (M/50 and M/100) but does occur in higher concentrations (M/20, M/10, and M/5). It shows further that in paramecia not adapted to a given monovalent cation salt ciliary reversal occurs in all concentrations from M/100 to M/5 and that the duration of reversed ciliary action in a given salt concentration is greater in nonadapted than in adapted specimens. These facts show that it is the rate of change (increase) and not absolute concentration of monovalent cation salt in the environment that induces reversal in the action of the cilia.

THE EFFECT OF TEMPERATURE ON CILIARY REVERSAL

Strasburger (1878) found that swarm spores which are photopositive in a given light intensity at 16°-18° C. become negative when the temperature is decreased to 4° C.

and more strongly positive when it is increased to 35° C. Massart (1891) obtained similar results in observations on *Chromulina* and Mast (1911) in observations on *Euglena*, *Chlamydomonas*, *Trachelomonas*, *Chlorogonium*, and *Volvox*; but no observations have been made on the effect of change in temperature on reversal in ciliary action.

In making this investigation, the temperature was controlled within 0.5° C. by means of temperature chambers on the stage of a low-power binocular microscope (Pitts, 1932). In making the tests, paramecia were thoroughly washed and then left 1½ hours in spring water at a given temperature in a temperature chamber on the stage of a binocular microscope. One individual was then taken up in a capillary pipette and put into 5 cc. of solution to be tested (in another temperature chamber on the stage of another micro-

TABLE 2

EFFECT OF ADAPTATION TO SALT ON DURATION OF REVERSED CILIARY ACTION IN *Paramecium*.

Each number in the table is the average of the duration in seconds of reversal for ten specimens. *Ins.* indicates reversal too short in duration to be measured with a stop watch.

CONCENTRATION	AVERAGE DURATION IN SECONDS OF REVERSED CILIARY ACTION								
	NaI	NaBr	NaCl	LiI	LiBr	LiCl	KI	KBr	KCl
Paramecia adapted to M/40 Salt Solutions									
M/100.....	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M/50.....	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M/20.....	1.24	0.75	1.29	2.10	4.62	4.02	24.15	5.49	1.39
M/10.....	2.47	2.67	2.51	5.07	8.33	7.00	41.62	13.91	6.60
M/5.....	6.41	3.82	7.29	5.10	23.79	17.67	55.17	16.01	9.77
Paramecia Not Adapted									
M/100.....	Ins.	Ins.	2.07	2.59	3.33	4.13	3.21	2.19	2.11
M/50.....	2.22	2.87	4.62	5.46	6.18	6.33	26.14	8.16	8.56
M/20.....	5.10	4.15	6.18	12.17	11.23	8.45	43.52	28.14	24.34
M/10.....	8.27	9.11	8.76	15.24	13.82	13.27	51.14	37.64	32.67
M/5.....	11.31	11.27	11.39	12.00	18.10	7.53	73.46	45.58	41.21

scope) at any temperature desired, then the behavior of the specimen was observed continuously until the end of the experiment. If there was ciliary reversal, the duration was measured with a stop watch. This procedure was repeated as often as desired. Observations were made on ten specimens at each temperature tested.

Paramecia were left in spring water at 23° C. for 1½ hours, then transferred to spring water at 7°, 15°, 23°, 30°, 40°, and 50° C. The following was observed: at 7°-30° there were violent motor reactions but no continuous backward swimming. At 40° C. there was ciliary reversal which continued 1.5 seconds. At 50° the paramecia died immediately. In paramecia adapted to 15° and 30°, respectively, similar results were obtained. The paramecia did not become adapted to 7°, 35°, and 40° C.

These experiments indicate that reversal in ciliary action occurs only after transfer to temperatures almost immediately lethal and then continues only a very short time.

Paramecia were adapted for $1\frac{1}{2}$ hours to spring water at 23°C . then put into 5 cc. of various chemical solutions at 7° , 15° , 23° , 30° , 40° , and 50°C . The results obtained are presented in Table 3.

Table 3 shows that duration of reversed ciliary action in M/20 and M/50 solutions of all the salts tested varies inversely with temperature and that in a given concentration of chloride, bromide, or iodide of potassium, sodium, and lithium at a given temperature the order of effectiveness of the monovalent cations in inducing reversal is $\text{K} > \text{Li} > \text{Na}$.

TABLE 3

RELATION BETWEEN DURATION OF REVERSED CILIARY ACTION IN *Paramecium*
IN M/20 AND M/50 SOLUTIONS OF SALTS

Each number in the table represents the average duration of reversal in seconds in ten specimens. Death was immediate without reversal.

SALT	CONCENTRATION	TEMPERATURE					
		7°C .	15°C .	23°C .	30°C .	40°C .	50°C .
KCl.....	M/20	68.63	42.09	28.20	20.36	16.81	Death
KCl.....	M/50	48.12	20.86	11.58	6.25	3.80	"
LiCl.....	M/20	77.31	32.80	8.94	4.89	3.71	"
LiCl.....	M/50	65.91	24.14	6.57	3.69	2.23	"
NaCl.....	M/20	23.29	11.79	5.60	2.80	1.61	"
NaCl.....	M/50	18.94	9.53	4.22	1.86	1.24	"
KBr.....	M/20	83.89	52.44	27.39	19.53	8.29	"
KBr.....	M/50	39.27	21.63	12.15	4.81	3.20	"
LiBr.....	M/20	78.33	29.56	11.72	5.32	4.18	"
LiBr.....	M/50	51.12	19.27	6.52	5.15	4.78	"
NaBr.....	M/20	22.89	11.91	5.37	3.99	1.76	"
NaBr.....	M/50	17.62	9.21	4.47	2.26	2.15	"
KI.....	M/20	97.30	44.21	30.76	12.92	8.22	"
KI.....	M/50	43.97	16.81	11.21	5.38	3.36	"
LiI.....	M/20	87.49	31.20	13.18	5.26	3.67	"
LiI.....	M/50	44.34	16.51	7.16	2.94	2.72	"
NaI.....	M/20	35.62	15.41	7.06	4.39	2.69	"
NaI.....	M/50	26.43	9.27	4.21	3.53	2.47	"

Comparison of relation between duration of reversal in a given concentration of chlorides, bromides, and iodides having the same cation and temperature indicates that, if these anions affect ciliary reversal, there is no constant or significant order in which they affect it. The similarity of effects produced by salts of the same cation but different anions is particularly striking in the salts of lithium given in Table 3.

There were marked differences in rate at which paramecia swam backward at different temperatures. The rate was very slow at 7°C ., somewhat more rapid at 15°C ., rapid at 23° , very rapid at 30° , and extremely rapid at 40°C . Actual measurements were not made, but careful observation indicates that it is probably directly proportional to the temperature.

Paramecia exposed to the lower temperatures became darker in appearance and more vacuolated, and the granular nature of the endoplasm was more pronounced. Death in high temperatures was accompanied by increased vacuolation change in shape and coagulation of the cytoplasm.

Similar results were obtained with paramecia adapted to 15° and 30°C .

DISCUSSION

It is demonstrated above that *Paramecium* reacts to increase in monovalent cation salt concentration in the environment by reversal in action of the cilia but does not react to increase in bivalent cation salt concentration. This difference in response must be due to some difference in the effect of monovalent and bivalent cations on the protoplasm.

Heilbrunn (1923) found chlorides of calcium and magnesium cause a decrease, while chlorides of potassium, sodium, and ammonium cause an increase in protoplasmic viscosity of eggs of *Arbacia*. He (1923) obtained similar results with *Stentor*, as did Heilbrunn and Daugherty (1931) in *Amoeba dubia*. Cholodnyj (1923) found potassium and lithium produce marked increase in protoplasmic viscosity in root hairs of *Trianea* but that calcium and magnesium have no appreciable effect. Weber (1924) reached similar conclusions in reference to effects of sodium and calcium chloride on *Spirogyra*, and Spek (1921) maintains that viscosity of the protoplasm in *Actinosphaerium* is increased by cations in the following order: $\text{Li} > \text{K} > \text{Na} > \text{Ca} > \text{Mg}$. Spek (1920) found LiBr , LiCl , and KOH cause *Paramecium* to increase in volume, and he attributes this to a swelling action of the colloid resulting in increased viscosity. There is consequently, considerable evidence which indicates that monovalent cations induce increase and bivalent cations decrease in viscosity of protoplasm.

If this is true and the cilia are under control of the neuromotor apparatus, it is conceivable that an increase in viscosity of the cytoplasm could produce impulses in the neuromotor apparatus resulting in reversal in the action of the cilia.

That reversal is associated with increased viscosity is further suggested by the observation that reversal in response to temperature occurs only at temperatures almost immediately lethal, it being well established that there is a marked increase in viscosity preceding death at high temperatures. It is also shown that duration of reversal induced by chemicals varies inversely with temperature, and there are many observations which indicate that viscosity of protoplasm varies inversely with temperature.

Precisely how environmental changes which have been observed to induce reversal in the action of cilia effect reversal and the physiological factors involved cannot be completely explained on a basis of our present knowledge. Factors such as changes in the electric potential at the surface, or in permeability and consistency of the surface layer of the organism, or chemical changes in the receptors or elsewhere in the organism may be involved as has been suggested. It is believed, however, that there is considerable evidence to indicate that reversal is associated with increase in viscosity of the cytoplasm. A more comprehensive study of this problem is being undertaken.

SUMMARY

1. The chlorides, bromides, and iodides of potassium, lithium, sodium, and ammonium induce reversal in the direction of the effective beat of the cilia of *Paramecium*. The chlorides, bromides, and iodides of calcium and magnesium do not.

2. The duration of reversed ciliary action varies directly with concentration of the salt which induces it.

3. The effect of monovalent salts on reversal is primarily due to the action of the cations. If the anions have any effect, it is quantitative and is secondary to that of the cations.

4. The order of effectiveness of the monovalent cations in inducing reversal is $\text{K} > \text{Li} > \text{Na} > \text{NH}_4$.

5. *Paramecia* which have been 18 hours in a given concentration of chloride, bromide, and iodide of potassium, lithium, and sodium become adapted and do not exhibit ciliary reversal when transferred to solutions of lesser concentration but do reverse to solutions of higher concentration. Duration of reversal in adapted is less than in nonadapted specimens. Ciliary reversal is dependent upon rate of increase in concentration of monovalent cation salt in the environment.

6. The order in which the monovalent cations produce death in *Paramecium* is $Li > K > Na$.

7. Prolonged exposure to M/40 lithium and potassium salts frequently results in change in direction of rotation on the longitudinal axis in forward locomotion and active changes in shape during locomotion.

8. Change in temperature which is not lethal does not induce reversal in direction of the effective beat in the cilia in *Paramecium*.

9. Lethal temperatures induce reversal in the action of the cilia if death is not immediate, but the duration of the reversed action is very short.

10. The duration of reversed ciliary action in solutions of monovalent cation salts varies inversely with temperature.

11. The order of effectiveness of monovalent cations in inducing reversal is independent of temperature.

12. The rate at which the cilia of *paramecia* beat in reverse varies directly with temperature.

LITERATURE CITED

- CHOLODNYJ, N. 1923. Zur Frage über die Beeinflussung des Protoplasmas durch mono und bivalente Metallen. Beihefte Bot. Centralbl., 39: 231.
- EISENBERG-HAMBURG, E. 1932. Einfluss der Sr-Salze auf die Bewegung von *Paramecium caudatum*. Arch. f. Protistkunde, 77: 108.
- HEILBRUNN, L. V. 1923. The colloid chemistry of protoplasm, I: General considerations; II: The electrical charges of protoplasm. Amer. Jour. Physiol., 64: 481.
- HEILBRUNN, L. V., and DAUGHERTY, K. 1931. The action of sodium, potassium, calcium, and magnesium on the protoplasm of *Amoeba dubia*. Physiol. Zööl., 4: 635.
- JENNINGS, H. S. 1899a. Studies on reactions to stimuli in unicellular organisms, II: The mechanism of the motor reactions of *Paramecium*. Amer. Jour. Physiol., 2: 311.
- . 1899b. Studies on reactions to stimuli in unicellular organisms, IV: Laws of chemotaxis in *Paramecium*. *Ibid.*, 355.
- MASSART, J. 1891. Recherches sur les organismes inférieurs. La Sensibilité à la concentration chez les êtres unicellulaires marins. Bull. Belg. Acad., Ser. 3. 22: 148.
- MAST, S. O. 1911. Light and the behavior of organisms. New York. Pp. 410.
- MAST, S. O., and NADLER, J. E. 1926. Reversal in ciliary action in *Paramecium caudatum*. Jour. Morph. and Physiol., 43: 105.
- MERTON, H. 1923. Studien über Flimmerbewegung. Arch. f. d. ges. Physiol. 198: 1.
- . 1935. Zwangsreaktionen bei *Stentor* als Folge bestimmter Salzwirkungen. Biol. Zentrabl., 55: 268.
- PARKER, G. H. 1896. The reactions of *Metridium* to food and other substances. Bull. Mus. Comp. Zööl., 29: 107.
- . 1905a. The reversal of ciliary movement in metazoans. Amer. Jour. Physiol., 13: 1.
- . 1905b. The reversal of the effective stroke of the labial cilia of sea anemones by organic substances. *Ibid.*, 14: 1.
- PITTS, R. F. 1932. The relation between hydrogen ion concentration, inorganic salt concentration, and rate of locomotion in *Amoeba proteus*. Johns Hopkins University dissertation. Pp. 191.
- SPEK, J. 1920. Experimentelle Beiträge zur Kolloid-Chemie der Zellteilung. Kolloidchem. Beihefte, 12: 1.
- . 1921. Der Einfluss der Salze auf die Plasmokolloide von *Actinosphaerium Eichorni*. Acta. Zool. (Stockholm), 2: 153.
- STRASBURGER, E. 1878. Wirkung des Lichtes und der Wärme auf Schwärmsporen. Jena Zeitsch., N.F., 12: 551.
- WEBER, F. 1924. Plasmolyseform und Protoplasmaviskosität. Österreich Bot. Zeitsch., 73: 261.

VITA

Joseph F. Oliphant was born in Farmersburg Indiana on July 31, 1906. He received his elementary and secondary education in the public schools of Farmersburg. He received the A.B. degree from Wabash College in June, 1928. He entered the Johns Hopkins University as a student in Zoölogy in the fall of 1928 and received the Ph.D. degree in June, 1935. During the years 1929-31 he was instructor in Biology in Union College.

